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FLORAL BIOLOGY OF *ADOLPHIA INFESTA* AND THE REPRODUCTIVE PROFILE OF COLLETIEAE (RHAMNACEAE)

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Abstract—The reproduction of the shrub *Adolphia infesta* was studied in a population included in a Mexican agricultural landscape with small, cultivated plots and fragments of natural habitat. *Adolphia infesta* had reproductive traits consistent with those known for its tribe Colletieae, including small zoophilous flowers exposed to both xenogamous, geitonogamous and visitor-facilitated intrafloral pollination, with selfing being at least partially hindered by self-incompatibility. Visual and olfactory cues, together with easily accessible rewards (nectar and pollen), explain the diversity (68 species of 29 families) of insects attracted to the flowers. However, only a small subset can be considered potential pollinators (honeybee and three muscoid flies). In late floral development, pedicel bending brings the developing fruit to an upright position, which might later optimise explosive seed dispersal. The finding of this trait in *A. infesta* makes fruit erection a synapomorphy of the *Adolphia – Discaria – Kentrothamnus* clade of the tribe Colletieae. At the study site, *A. infesta* played a significant role as food source for the local anthophilous insect community, and through its flower visitors, the plant was indirectly connected to 11 other plant species. Knowledge derived from community studies should be applied in conservation initiatives directed at enhancing farmers' appreciation of extant local biodiversity

Keywords: Flower longevity, pollinator exposure, pollen limitation, stochastic pollination environment

INTRODUCTION

The tribe Colletieae (Rhamnaceae) has seven genera (Medan & Schirarend 2004, Aagesen et al. 2005, Kellermann et al. 2005). Studies of their reproductive biology have produced information about 16 out of the tribe's nineteen species (references in Medan et al. 2013, Medan & Torretta 2014). Their generalised reproductive traits include many small, coloured, relatively short-living entomophilous flowers that open during a single annual flowering episode (Medan & Torretta 2014). This extends over several weeks, allowing for both xenogamous and geitonogamous pollination, with selfing being at least partially hindered by protandry and self-incompatibility (Medan & Torretta 2014). Complexity is added by (a) trait deviations occurring at specific or generic level, like ornithophily (*Colletia ulicina*, Medan & Montaldo 2005), homogamy (*Kentrothamnus,* Medan et al. 2013; *Colletia*, Medan & Torretta 2014), late flowering (*Colletia*, Medan & Torretta 2014), and ambophily (*Discaria trinervis*, Medan & Devoto 2005); and (b) existence of various advertisement, reward, and seed dispersal strategies across genera. In this context, a study of *Adolphia* (the only genus still reproductively unknown in the Colletieae) was necessary before a more complete analysis of the evolution of reproductive strategies in this tribe could be undertaken. The reproduction of *Adolphia* was particularly intriguing in view of its isolated distribution in North America, far apart from its Southern Hemisphere relatives (Tortosa 1993, Aagesen et al. 2005). *Adolphia* is regarded as monotypic (Tortosa 1993).

This paper focuses on the reproduction of *Adolphia infesta* (Kunth) Meisn. in a population located in the state of Veracruz, México. Here, *A. infesta* occurs in the intermontane valleys, either on terrain strips left uncultivated to serve as windbreaks or in adjacent rocky areas only suitable for cattle grazing. As the study involved identification of the plant's animal pollinators, it was also expected to provide an insight into the richness of the flower–animal interactions supported by the local agroecosystem. Specifically, we identified the main reproductive traits of *A. infesta* with respect to reproductive phenology, flower biology and breeding system. We then discussed whether these traits are consistent with those already known for other members of Colletieae, the tribe in which *Adolphia* is included, and finally, identified the flower visitors and co-flowering plant species interacting with *A. infesta* during its blooming.

MATERIALS AND METHODS

Focal species

Adolphia infesta grows in the US and México between 200 and 2,600 m a.s.l. as a spiny, nitrogen-fixing, virtually leafless shrub that, depending on altitude and exposition, reaches a height of 0.5 to 3 m at reproductive age (Cruz Cisneros & Valdés 1991, Tortosa 1993) (Fig. 1A, C).

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FIGURE 1. *Adolphia infesta.* (A) habit, (B) a flowering node displaying mid- and late-anthesis flowers, (C) a patch consisting of several individuals, with cultivated plots and uncultivated windbreaks at the background, (D to H) flowers in anthesis stages 2, 3, 4, and 5 (D to G; note that stage 2 is represented by a 4-merous flower) and a developing fruit (H). Arrows in (D) and (F) indicate petals. Bar = 15 cm (A), 2 mm (B), 1 m (C) and 2 mm (D, also valid for E-H). Photos by D. Medan.

Flowers are presented 3-7 together in cymes grouped in inflorescences, which usually resume vegetative growth after anthesis (Tortosa et al. 1996). Flowers are pedicellate, hermaphrodite, actinomorphic, 4-5-merous, 4 mm in diameter, and with a 1 mm long × 2.5 mm wide, cup-shaped, externally white–reddish floral tube (Medan 1985). After anthesis the floral tube persists as a fruit pedestal (Medan & Aagesen 1995). The deltoid sepals and the petals/stamens are alternate. Anthers are raised 0.5 mm above the opening of the floral tube. An adpressed-type nectary lines the inside surface of the floral tube (Medan & Aagesen 1995). The 3-4-carpellate gynoecium is composed of a semi-inferior, 3-4-ovulate ovary, a style, and a terminal stigma located at anther height. Fruits are 1-4-seeded explosive capsules.

Study site

We worked in July 2013 on a population located 5 km west of Tenextepec, municipio Perote, state of Veracruz, México (19°27'59" N, 97°18'22" W, 2,380 m a.s.l.). Here *A. infesta* grows along 10 m wide, parallel strips intercalated between 30 m × 500-800 m maize or bean plots (Fig. 1C, background). These strips are left uncultivated to serve as windbreaks against summer winds. Plants of *A. infesta* are also found on the higher, rocky terrain, the *malpaís* (Fig. 1C, foreground), adjacent to cultivated plots. Goat herds are brought to these areas and along the windbreaks for grazing. Natural vegetation on windbreaks and *malpaís* is dominated by *Juniperus flaccida* Schltdl. (Cupressaceae), *Pinus cembroides* Zucc. (Pinaceae), *Nolina parviflora* (Kunth) Hemsl., *Yucca elephantipes* var. *ghiesbreghtii* Molon, and *Agave americana* L. (Asparagaceae). At the time of the study, the flowering zoophilous plant community included fifteen species of ten families. Bushes of *A. infesta* were often associated with *Opuntia huajuapensis* Bravo (Cactaceae) and *Croton dioicus* (Euphorbiaceae), and offered refuge to vertebrate wildlife, notably the venomous rattlesnake *Crotalus ravus* Cope. Climate is semiarid, with an annual rainfall of ca. 525 mm. During fieldwork, temperature and wind speed were measured. For further information on climate, soil, and vegetation of the study area see Ramos & González (1972), Soto et al. (1977), and Soto & García (1989).

Study material

We randomly selected twenty *A. infesta* patches for study. Individual patches covered 41.8 ± 40.4 m² (mean ± 1 S.D.; range 1-160 m², whole patch area = 836.5 m²), included a variable number of *A. infesta* individuals, and were located both on the windbreaks and in adjacent

malpaís. Voucher specimens of the study species are kept at the Gaspar Xuarez herbarium (BAA, University of Buenos Aires, Argentina).

Reproductive phenology and pollination biology

The population's overall phenological status was assessed by comparing counts of branches with open flowers with counts of branches showing only buds, in representative 1 m^2 quadrats of all patches. To define flower phenological stages, twenty-five flower buds and open flowers from different individuals were dissected and inspected under a 30× stereomicroscope in the field. Additionally, eleven flowers from three individuals were separately labelled and monitored from the unopened bud stage until anthesis was completed, in order to define floral morphological stages according to the position of sepals and petals, and the status of pollen, stigma, nectar and scent presentation (Tab. 1). Stigmatic receptivity was assessed with the hydrogen peroxide test (Dafni et al. 2005). Although present in all flowers, nectar failed to accumulate in sufficient amounts to permit extraction, even using very thin handmade microcapillaries.

Flower visitors were observed and sampled across the population on five days and at different times of day between 9:00 and 18:00 hrs (cumulative time = 20 h), in order to record the entire visitor community. An insect was recorded as a flower visitor when it was possibly in contact with anthers or stigmas. One hundred and sixty-nine insect individuals were caught for identification, and their number was used as a measure of each species' abundance. Sixty-seven percent of the visitor taxa were identified to genus or species. When identification to species was not possible, the specimens were morpho-typed. All collected material is deposited at the Entomological Collection of the General Botany Unit (FAUBA), School of Agronomy, University of Buenos Aires. On July 10th, a 10-min visitor census was conducted at each of five plants. Each censused area included on average 140 open flowers. For the study of pollen loads carried by insects, all collected individuals were inspected under 50× magnification. After excluding corbicular pollen, considered to be too tightly packed to be available for later deposition on flowers, pollen loads (including pollen both of the study plant and of other species) were ranked as 3 = large pollen masses present, 2 = 100-150 grains in small but easily visible groups, 1 = less than 20 widely scattered grains, and 0 = no visible grains. Ranks assigned to individuals were averaged to obtain values of pollen transportation ability for each insect species. In species scored with value = 1 or higher, one or two representative individuals were chosen for microscopic identification of pollen taxa (N = 65 individuals). Pollen was removed from selected individuals by gently rubbing pollen-loaded surfaces with 1 mm3 portions of safranin-stained gelatine jelly. These gelatine cubes were then melted on glass slides, covered and observed under microscope for pollen. Pollen grain identification included a comparison with a reference collection of slides from authentic samples taken from flower buds collected in the field. The number of "pollen taxa" found in the loads obtained from all specimens of a species was used as a measure of the insect species' generalization. For each insect species, a simple pollination effectiveness index was computed as E = number of insect individuals collected and observed × pollen transportation ability (see above) / generalization. Since the index increases with insects' abundance, size and purity of the transported pollen load, it expresses each visitor species' relative contribution to pollination of *A. infesta*. Average E values for insect families and orders were also calculated. Values of abundance of visitor species deduced from censuses could not be used for calculation of E because only a small fraction of the visitors were recorded on censuses.

Breeding system and maternal success

To assess the relative effect of wind and insect visitors on fruit set, an experiment was conducted involving three treatments that were applied to one randomly selected individual per patch (N = 20 plants). (1) *Open pollination*: Flower buds were left uncovered and experienced natural pollination (N = 11,336 flowers). (2) *Mesh bagging:* Flower buds were covered with 1 mm-mesh bridal veil bags to prevent insect visitation while enabling wind pollination (N = 5,092 flowers). (3) *Cloth bagging:* As in 2, but using white muslin bags, excluding both insects and airborne pollen (N = 6,190 flowers). Occasional observations indicated that neither mesh bagging nor cloth bagging changed flower phenology, suggesting that bagging did not affect floral viability in either treatment. Experimental branches were collected 30 d after the start of the experiment, when all flowers had abscised or were developing into fruit. Per-plant fruit-to-flower ratios were computed and analysed with Kruskal-Wallis and Bonferroni-corrected Median tests.

To assess the relative effect of outcross pollen on fruit set, a hand-pollination treatment was applied to a subset of three randomly selected plants. In experimental branches bagged as in treatment (3) above, all open flowers (except flower buds expected to open on the next few days) were removed from inside the closed bags. Two days later the bags were opened and flowers with abundant stigmatic secretion (considered as an indication of stigmatic receptivity) were selected for manipulation. With the aid of a 10× hand lens, stigmas were visually controlled for lack of self pollen and pollinated by gently touching them with an anther of a freshly opened flower of another individual located at least 10 m distant from the receptor individual. Afterwards, bags were closed again. Two days later, flowers were collected, fixed in FAA, transported to the laboratory, soaked for 48 h in 5% NaOH (w:v) at 30ºC, cleared in diluted NaClO, mounted on 0.1% decolourised aniline blue, and viewed with a fluorescence microscope (Martin 1959) (N = 23 flowers). Untreated, open-pollinated flowers of the same three experimental plants were similarly processed (N = 27 flowers). For each flower, the number of ovules penetrated by pollen tubes was recorded. On a per-plant basis, proportions of crossed vs. open-pollinated flowers with penetrated ovules were compared with a Kruskal-Wallis test.

For a further assessment of the size of stigmatic loads under open pollination, samples of old open flowers from a random ten-individual subset of the plants used in the

pollination experiment were collected, air-dried and transported to the laboratory. On a microscopic slide, each stigma was macerated for 24 h in a drop of 10% NaOH (w:v). Then a small volume of melted safranin-stained glycerine jelly was added, and the stigma was gently squashed under a cover slip and inspected microscopically to count pollen grains (N= 141 flowers from ten individuals).

To quantify the total floral display in experimental patches, the number of open flowers was counted in three 0.25 m² quadrats in each of three randomly selected patches. The nine counts were averaged and the final value was multiplied by the area of each patch. To have an estimate, albeit conservative, of the number of flowers produced during the entire reproductive season, counts of flowers in the 20 control branches from the breeding system experiment were averaged. This figure was first multiplied by 5 (a conservative estimation of the number of flowering branches/m²) and then by the area of each patch.

RESULTS

Phenology and flower biology

The proportion of reproductive branches with open flowers showed that the population was in the first third part of its flowering. Individuals at different phenophases coexisted (e.g., plants opening their first flowers and plants already ripening fruit) indicating large inter-individual variation in blooming start date. The size of the simultaneous floral display was ca. 560 open flowers per m2, resulting in ca. 272,000 flowers in all patches. Patches were estimated to produce ca. 2,800 flowers/m² during the entire reproductive season, equivalent to 1,363,000 flowers for the whole patch set.

A typical flowering node displayed ca. 4 open flowers at the same time (Fig. 1 B). Flowers started anthesis at different times of the day and had pollen export and receipt for 2 days. Six distinct floral stages were identified (Fig. 1 D-H, stages 1-6 in Tab. 1).

At day 1 (stage 1), sepals were closed but anthers were already dehisced, while stigma and nectary were still dry. At day 2 (stages 2 and 3, Fig. 1D-E) sepals opened and gradually spread, pollen removal by insect visitors started (although the stamen-opposed, hooded petals limited access to anthers from above), nectar secretion started, and a mildly fetid smell was perceptible. At day 3 (stage 4, Fig. 1F) sepals became horizontal (i.e. transversal to floral axis), while nectar secretion and smell production continued. Petals started spreading, which increased anthers' exposure to visitors, and the stigma became swollen and wet, showing a clear reaction to the peroxidase test (a few flowers had reacted weakly to this test at stage 3). Both cross-pollination and visitor-facilitated intrafloral self-pollination were possible at stage 4. At day 4 (stage 5, Fig. 1G) petals, stamens and style started to wilt, nectar secretion and smell production stopped, and visitation ceased, while the ovary colour changed from green to red. Starting at day 5 (stage 6) the superior part of the ovary enlarged, both the floral tube and the pedicel became thicker, and the pedicel curved upwards, driving the developing fruit to an upright position (Fig. 1H). All other floral parts withered and finally dropped.

Pollinators and pollination service

Visits of insects to flowers occurred between 9:00 and 16:00 hrs, at temperatures around 18°C or higher and breezes up to 1.2-1.5 m/s strong. On most days around 3-4 p.m., the weather became cloudy and cooler, some rainfall occurred, and insect activity stopped.

TABLE 1. Flower phenology of *Adolphia infesta*.

Floral part / floral stage	Day 1 Stage 1	Day 2 Stage 2	Stage 3	Day 3 Stage 4	Day 4 Stage 5	Day 5 onwards Stage 6
Sepals	closed	open and erect	spreading to horizontal	horizontal	horizontal	wilting
Petals	concealing anthers	partially concealing anthers	partially concealing anthers	starting spreading, thus increasing anthers' exposure	spread and wilting	spread and wilting
Stamens	anthers open	anthers still connivent but pollen available	filaments erect, pollen partly removed	starting spreading, pollen mostly removed	spread and wilting, pollen fully removed	spread and wilting, then dropped
Pistil	stigma dry, peroxidase test negative	stigma dry, slightly below anther height, peroxidase test negative	stigma somewhat swollen, at anther height, peroxidase test faintly positive	stigma swollen, wet, slightly over anther height, peroxidase test positive	stigma and style wilting, ovary changing from green to red	stigma and style dropped, ovary enlarging
Pedicel	orientation variable	orientation variable	orientation variable	orientation variable	orientation variable	becomes erect
Nectar	none	present	present	present	none	none

TABLE 2. Identity, abundance (cumulative number of records during censuses, and number of collected individuals), pollen transportation ability (average size of body pollen load; for details on scale definition see Material and Methods), generalization (number of plant species represented in the pollen load), and pollination effectiveness (abundance × pollen transportation ability/generalization) of insect visitors to *Adolphia infesta* flowers. Average effectiveness values for insect families and orders are also provided. Putative pollinators (visitor species showing medium to high abundance and effectiveness values) appear in **bold type**. Abbreviations: C: Coleoptera, D: Diptera, HE: Hemiptera, HY: Hymenoptera, L: Lepidoptera.

Order	Family	Species	Abundance				Effectiveness		
			census records	nr. collected specimens	pollen transportation ability	generalization	species	family average	order average
C	Scarabaeidae	*Macrodactylus nigripes* Bates	-	3	2	1	6	6	11
C	Lycidae	*Lycus schönherri* Chevrolat	3	8	2	1	16	16	
D	Agromyzidae	cf. *Melanagromyza* sp.	-	1	1	2	0.5	0.5	1.6
D	Bibionidae	*Plecia nearctica* Hardy	-	4	1.2	2	2.4	2.4	
D	Bombyliidae	*Villa* sp.	-	1	1	3	0.3	0.3	
D	Calliphoridae	***Eucalliphora* sp.**	48	12	0.7	2	4.2	1.8	
D		*Paralucilia* sp.	-	1	2	2	1		
D		*Phaenicia* sp.	-	2	2	2	2		
D		*Phormia regina* (Meigen)	-	1	2	4	0.5		
D		unidentified sp.	-	1	1.5	1	1.5		
D	Heleomyzidae	*Pseudoleria* sp.	-	1	0	0	0	0	
D	Muscidae	***Musca domestica* L.**	23	9	1.7	2	7.6	4.3	
D		unidentified *Muscini* sp.	-	1	1	1	1		
D	Anthomyidae	unidentified sp.	-	1	0	0	0	0	
D	Sarcophagidae	unidentified sp. 1	3	1	2	1	2	0.8	
D		unidentified sp. 2	-	1	1	2	0.5		
D		unidentified sp. 3	-	1	1	1	1		
D		unidentified sp. 4	-	1	1	2	0.5		
D		unidentified sp. 5	-	1	0	0	0		
D	Stratiomyidae	*Euryneura mexicana* Kertész	-	1	1	2	0.5	0.5	
D	Syrphidae	*Allograpta neotropica* Curran	-	1	1	4	0.25	1.75	
D		*Allograpta obliqua* (Say)	-	8	1	1	8		
D		*Copestylum fornax* (Townsend)	-	3	1	3	1		
D		*Copestylum marginatum* (Say)	1	14	1.9	3	8.9		
D		*Copestylum melleum* Jaennicke	-	1	2	3	0.7		
D		*Copestylum* sp.	-	2	0	0	0		
D		*Eristalis tenax* (L.)	-	1	2	3	0.7		
D		*Eristalis stipator* Osten Sacken	-	1	2	3	0.7		
D		*Helophilus* sp.	-	1	2	1	2		
D		*Lejops arquatus* Say	-	2	1	5	0.4		
D		*Lejops polygrammus* Loew	-	1	1	5	0.2		
D		*Palpada vinetorum* Fabricius	-	1	2	4	0.5		
D		*Palpada* sp.	-	1	2	3	0.7		
D		*Sphiximorpha* sp.	-	5	0.2	2	0.5		
D	Tachinidae	*Adejeania* sp.	-	3	1.3	1	3.9	1.37	

TABLE 2. ctd.

Order	Family	Species	Abundance				Effectiveness		
			census records	nr. collected specimens	pollen transportation ability	generalization	species	family average	order average
D	Tachinidae	unidentified sp. 1	-	5	1.6	2	4		
D		unidentified sp. 2	-	1	1	2	0.5		
D		unidentified sp. 3	-	2	1	2	1		
D		unidentified sp. 4	-	2	0	0	0		
D		unidentified sp. 5	-	1	2	3	0.7		
D		unidentified sp. 6	-	1	2	4	0.5		
D		**unidentified sp. 7**	14	6	1	2	3		
D		unidentified sp. 8	-	1	0	0	0		
D		unidentified sp. 9	-	1	1	2	0.5		
D		unidentified sp. 10	-	1	2	2	1		
D	Ulidiidae	*Tetanops* cf. *myopaeformis* Röder	7	3	1.7	1	5.1	5.1	
HE	Largidae	*Largus* sp.	-	7	2	1	14	14	14
HY	Andrenidae	*Andrena* sp.	-	4	1.1	3	1.5	1.5	1.6
HY	Apidae	***Apis mellifera* L.**	97	3	2	1	6	2.25	
HY		*Doeringiella (Triepeolus)* sp.	-	1	1	2	0.5		
HY		*Eulaema polychroma* (Mocsáry)	-	1	1	4	0.25		
HY	Colletidae	*Colletes* sp.	-	2	2.5	2	2.5	2.5	
HY	Crabronidae	*Philanthus* sp.	-	1	2	3	0.7	0.7	
HY	Halictidae	*Lasioglossum (Dialictus)* sp. 1	-	2	1.5	2	1.5	2.25	
HY		*Lasioglossum (Dialictus)* sp. 2	3	7	1.3	3	3		
HY	Ichneumonidae	*Compsocryptus calypterus* (Say)	-	2	2	1	4	4	
HY	Pompilidae	*Pepsis* sp. 1	-	1	2	4	0.5	0.9	
HY		*Pepsis* sp. 2	-	2	2	3	1.3		
HY	Scoliidae	cf. *Crioscolia* sp.	-	1	2	4	0.5	0.5	
HY	Sphecidae	*Bembix americana* Fabricius	-	1	1	2	0.5	0.5	
HY	Tiphiidae	unidentified sp.	-	1	2	4	0.5	0.5	
HY	Vespidae	cf. *Pseudodynerus* sp.	-	1	2	1	2	2	
L	Hesperiidae	*Polygonus leo* (Skinner)	-	1	0	0	0	0	0.5
L	Lycaenidae	*Theclinae* sp. 1	-	1	0	0	0	0	
L		*Theclinae* sp. 2	-	1	0	0	0		
L		*Theclinae* sp. 3	-	1	0	0	0		
L	Noctuidae	*Alypiodes bimaculata* (Herrich-Schäffer)	-	4	1	2	2	1.5	
L		*Cyanopepla gloriosa* Walker	-	1	2	2	1		

Sixty-eight species of insects belonging to twenty-nine families of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera were recorded as flower visitors (Tab. 2). Diptera and Hymenoptera dominated the visitor assemblage, representing 88% of the total number of species. Across the visitor assemblage, pollination effectiveness values ranged from $E = 0$ to $E = 16$. Flies, bees and wasps showed relatively low (and coincident) average effectiveness values ($E = 1.6$). In contrast, Coleoptera and Hemiptera accounted for only three species, but showed much higher effectiveness values ($E = 11$ and 14, respectively). Butterflies and diurnal moths had intermediate

diversity and showed the lowest effectiveness values (E = 0.5) because four of the six species of Lepidoptera transported no pollen at all.

Sixty percent of the species were collected only once (Tab. 2). Only nine species were relatively common as visitors (Tab. 2). Among these, *Apis mellifera* accounted for almost half of the 199 visits recorded during censuses, followed by the flies *Eucalliphora* sp. (48 visits), *Musca domestica* (23) and an unidentified tachinid species (14). These four visitor species were highly mobile and carried either pure *A. infesta* pollen loads (*A. mellifera*) or loads in which *A. infesta* pollen was mixed only with that from *Croton dioicus* (*Eucalliphora* sp., *Musca dosmestica* and the unidentified tachinid fly), and are therefore considered putative pollinators. Assuming constant visitation rates, five hours a day of exposure to insects, samples of 140 flowers per census, and a duration of anthesis of 2 days, a typical *A. infesta* flower would receive (199 visits / 0.83 h × 140 flowers × 5 censuses) × (5 hours of anthesis / day) × (2 days of anthesis) = 3.4 visits / flower over its lifespan, i.e. ca. 1.7 visits per day.

Breeding system and female success

The bagging experiment showed that pollination conditions affected fruit set (Kruskal-Wallis test for the whole experiment: H = 36.46, P < 0.0001) Percent fruit set was higher in open-pollinated flowers (18.9 ± 13.6) than in flowers covered with mesh bags (1.7 ± 1.6) and cloth bags (0.7 ± 2.0) (N = 20, 15 and 17 plants, respectively; Median tests, corrected after Bonferroni, P < 0.05).

In a ten-individual subset, stigmatic loads of open-pollinated flowers averaged 14.1 ± 13.1 (mean ± S.D) (N = 10) grains/stigma. In this subset, size of stigmatic loads and fruit set values under open pollination were positively and significantly correlated (Spearman rank correlation test, R = 0.3576, P < 0.05). No relationship was found neither between stigmatic load and area of the patch to which the individual belonged, nor between patch area and fruit set (R = 0.005 and 0.01, respectively).

In flowers inspected for pollen-tube performance, many tubes failed to grow farther than the stigma or the upper third of the style, particularly in untreated plants. A few pollen tubes grew down to the basis of the ovary and some penetrated the ovules. Hand cross-pollinated flowers showed ovule penetration more often than open-pollinated flowers in two out of the three tested individuals (100% vs. 27.3% and 100% vs 0.0% of the flowers, respectively), while in the third individual no penetrated ovules were recorded under either treatment. Even if average values diverged between treatments (66.6% vs. 9.1%, respectively) the difference did not reach statistical significance (Kruskal-Wallis H = 1.19, P = 0.4).

DISCUSSION

Adolphia and the reproductive profile of Colletieae

Adolphia infesta does not deviate from characteristics collectively considered as a reproductive "fingerprint" of Colletieae (Medan et al. 2013, Medan & Torretta 2014). As discussed below, these generalised traits include (1) the display of many entomophilous flowers during a single annual flowering episode, and (2) the occurrence of both xenogamous and geitonogamous pollination, with selfing being at least partially hindered by protandry and self-incompatibility.

(1) Advertising, rewards and pollinators

Adolphia infesta shows the tribe-dominant white floral colour (pure red flowers are exceptional, see Medan & Montaldo 2005), but its fetid floral scent (reflected in the local name *hierba hedionda*, stinking herb) is uncommon in the tribe. A similar unpleasant scent was reported in *Discaria americana*, a species sharing with *A. infesta* a fly-dominated flower visiting assemblage (Medan 1991). *Adolphia infesta* offers pollen and nectar as rewards for visitors (the usual trait shared by the group; see Medan et al. 2013 for the alternative high-pollen – low-nectar reward strategy found in a few members of the Colletieae tribe).

On a per-flower basis, the nectar amount offered to visitors is small, but the common presence of obligate nectar-feeders (butterflies and moths) as visitors of *A. infesta* indicates that this reward is consistently offered across flowers and individuals. On the one hand, nectar can be reached even by insect species with short mouthparts, because the flower tube is relatively shallow. The depth effect of *A. infesta* flowers is among the lowest in the tribe, equivalent to the ones in *Discaria pubescens* and *D. nitida* from Australia, and in *D. toumatou* from New Zealand (Medan & Aagesen 1995). On the other hand, easily accessible rewards help to explain the high diversity of the flower-visiting insect assemblage of *A. infesta*. This is not surprising, since only specialized flower morphologies tend to taxonomically restrict visitor arrays (Nilsson 1988, Moré et al. 2007, Bloch & Erhardt 2008). As pointed out in the Results' section, a large part of the flower visiting assemblage of *A. infesta* consists of uncommon, poorly mobile, promiscuous, and/or transport-inefficient species, and only four of these visitors sharing medium to high effectiveness, abundance and mobility were potential pollinators.

(2) Breeding system

Results lead to the conclusion that in *Adolphia infesta* (a) wind pollination is unimportant, (b) exclusion of flower visitors significantly lowers fruit set, and suggest that (c) access of pollen tubes to ovules triggers fruit initiation. Analysis of pollen tube behaviour suggested that outcross pollen tubes had preferential access to ovules, hence the possibility that some mechanism of self-incompatibility operates in this species should be further investigated. This mechanism has been shown to be common in the Colletieae (Medan et al. 2013, Medan & Torretta 2014).

Reproductive output seemed not primarily to be limited by pollen quantity, since on average flowers received 1.7 visits/day during two days, resulting in the deposition of 14.1 pollen grains/stigma, which is a sufficient load to deliver at least four pollen tubes to each ovule. However, fruit set did increase with larger stigmatic loads. Even if this

primarily suggests quantitative pollen limitation, qualitative limitation may also be involved. Since larger stigmatic loads may result from repeated visits, larger loads have higher chances of including outcross pollen. Thus, the positive relation between load size and fruit set may reflect low quality of pollen, originating from incomplete dichogamy and the simultaneous anthesis of many flowers in the same individual, which enable both intrafloral selfing and geitonogamy. Hence, every flower will receive some quantity of self, and presumably ineffective, pollen, which stresses the importance of insects as outcrossing agents. Aizen & Harder (2007) have shown that while the quantitative and qualitative components of pollen limitation are not easy to separate, quality limitation probably occurs much more often than generally supposed.

Pedicel erection in late floral development

Erection of the developing fruit was also reported for *Discaria americana* (Medan 1991) and *Kentrothamnus weddellianus* (Medan et al. 2013) among the Colletieae. Like *A. infesta*, these plants are low shrubs with explosive capsules. In explosively dispersed plants, maximum dispersal distance depends, among other factors, on the seed ejection angle (Swaine et al. 1979, Stamp & Lucas 1983, Witztum & Schulgasser 1995, Garrison et al. 2000), so it is conceivable that the upright position of the fruit has been selected to achieve farther dispersal. Note that in radially symmetrical fruits like those of *A. infesta* and its close relatives, all seeds (usually three) of a given fruit will be ejected at approximately the same angle *only* if the capsule is vertical at dehiscence. Optimal position of reproductive structures may differ between pollination and seed dispersal, and positional changes may avoid the conflict between these two processes (Webb 1984). Besides the mentioned examples in Rhamnaceae, pre-dehiscence changes in fruit position in ballistic seed dispersers have been reported for Euphorbiaceae (Garrison et al. 2000). In the Colletieae, the three genera displaying fruit erection (*Adolphia*, *Kentrothamnus* and *Discaria*) make up a single branch of the tribe's phylogenetic tree (Aagesen et al. 2005), thus this particular behaviour of the fruit stalk can be viewed as a synapomorphy that lends further support to this clade.

Biological interactions and conservation efforts

Habitat loss and reduction of the quality of the remaining habitat fragments menace many plant and animal species on a global scale, in particular where agriculture intensification is carried out (Millennium Ecosystem Assessment 2005, Aide et al. 2013). Areas where low impact agriculture and cattle raising are practised, like the Mexican landscape of the present research, deserve more intensive study to reveal to what extent the original local ecosystems are preserved. At our fieldwork site, flowers of *A. infesta* were visited by 68 species of insects, through which the focal plant indirectly interacted with 11 other plant species, i.e. over half of the co-flowering community. Even if these data are far from the picture that a fully resolved plant-pollinator network would provide, they still indicate that *A. infesta* played a significant role as food source for the local anthophilous insect guild. Community-scale studies are certainly needed to identify and measure all other pollination-associated interactions occurring in this agroecosystem, and efforts should be made to incorporate the resulting knowledge in conservation initiatives. For instance, the present attitude of local farmers and herders towards *A. infesta* is ambiguous, since the plant provides some forage for their goats, but it is also a hiding place for deadly *Crotalus* snakes, which often kill goats. The local appreciation of *A. infesta* could increase if it was shown that the plant helps to sustain the pollinators of other undisputedly useful species, like those preferentially consumed by goats.

Limitations of the study

Because only one population of *A. infesta* was examined and only during one reproductive episode, the conclusions of the present study should be treated with caution. Examination of additional populations could reveal between-site as well as between-year variation in reproductive traits. For instance, the pollination-generalist condition of *A. infesta* suggests that the composition of the visitor assemblage will show variations among sites and years, as well as across the species' geographical range. This caveat is counterbalanced by the fact that previous research on other species of the tribe Colletieae has shown only moderate between-population divergence when several populations were compared (Medan 1995, Medan & Devoto 2005, Medan & Montaldo 2005).

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